DRUG DISCOVERY

Biological evaluation of *Ficus elastica* leaves for antidepressant and anxiolytic activity

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ABSTRACT

Background: Medicinal plants play a vital role in questing of drug discovery as numerous of conjugates are present. Ficus plants, which are members of the Moraceae family, are frequently employed in traditional medicine to treat a wide range of illnesses. *Ficus elastica* is one of the plants used in Ayurveda for treatment of various CNS disorders. Recent statics of World Health Organisation (WHO) states that depression is one of the diversified neurological disorders and includes number of underlying diseases including anxiety, bipolar disorder etc. Ficus species are rich source of polyphenolic compounds, flavonoids which are responsible for psychiatric diseases such as neurodegenerative and hepatic diseases. *Results:* The methanol extract showed the significant decreased in the immobility time for FST (75.66 ± 3.38 sec and 52.66 ± 2.73 sec) and TST (91.0 ± 13.12 sec and 71.66 ± 14.4 sec) after 1 and 2 hr at 50 mg/kg dose. In elevate plus maze model the results described that chloroform extract (97.28 ± 3.70 sec) was significantly effective as compared to diazepam (106.53 ± 4.35 sec). Additionally, none of the extract displayed neurological toxic effect in rotarod test. *Conclusions:* The study revealed that *F. elastica* leaves consist of active phytochemical constituents which are responsible for the effectiveness against neurological disorders. The potent results of FST, TST, Elevated Plus Maze and Rotarod model of swiss albino mice confirms the antidepressant an anxiolytic activity of the *F. elastica* leave extracts. Different parts of *F. elastica* can be extracted evaluated for assessment of wide range of pharmacological activities. The results also encourage for the isolation of phytoconstituents from different ficus species and evaluation for the neurodegenerative diseases.

Keywords: *Ficus elastica*, Extraction, Antidepressant, Anxiolytic, Elevated Plus Maze, Rotarod, Immobility.

1. INTRODUCTION

Natural products have been utilising from ancient time to cure numerous diseases from life threating to mild diseases. There is significant number of the medications present in market today which is discovered from natural sources (Koehn and Carter, 2005). An ongoing area of study is still the hunt for novel naturally occurring substances that are physiologically active (Newman and Cragg, 2016). Two-thirds of the world's population in many nations, rely on

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herbal medicine as their primary form of healthcare. This fact accomplished about the social acceptability, compatibility and adaptation with the human body additionally they possessed fewer side effects as compared to the allopathy medicines (Cragg et al., 2014). The great level of biological diversity in nature is represented by a wide variety of chemical structures, some of which may be unmute molecules with stimulating biological properties. Such innovative natural compounds are frequently used as chemical building blocks in the development and production of novel pharmaceuticals (Mbosso-Teinkela et al., 2018).

Aspirin, which is based on the natural component salicin from *Salix alba* and morphine, which was derived from *Papaver somniferum*, were the first natural products to be marketed for medicinal use (Rosenblum et al., 2008; Dias et al., 2012). Numerous other plant-derived molecules reported as ground-breaking medications, including artemisinin from *Artemisia annua* used to treat malaria, capsaicin from *Capsicum annuum* used to treat pain, the two cannabinoids, dronabinol and cannabidiol from *Cannabis sativa* used, among other things, to treat chemotherapy-induced nausea and vomiting, *Atropa belladonna* produces tiotropium, an atropine derivative used as a bronchodilator in the treatment of chronic obstructive pulmonary disease, silymarin from the seeds of *Silybum marianum* and paclitaxel from *Taxus brevifolia* for treating lung, ovarian and breast malignancies (Schep et al., 2020; Alqahtani et al., 2019; Pinheiro et al., 2018; Atanasov et al., 2015).

Ficus plants, which are members of the Moraceae family, are frequently employed in traditional medicine to treat a wide range of illnesses (Trivedi et al., 1969). Since long time it is used as astringents, carminatives, stomachics, vermonicides, hypotensives, anthelmintics and anti-dysentery medications in traditional medicine (Abdel-Hameed, 2009). Numerous species of ficus, which are reproduced either by seeds or by cutting layers, are mostly found in the outer Himalayas, stretching from Nepal eastward to Assam and the Khasi Hills (Frodin, 2004). With over 800 species and 2000 different types of shrubs, herbs and woody trees, Ficus is one of the biggest genera of angiosperms (Salehi et al., 2021).

Ficus species belongs to Hemi-epiphytic plants which employ their host to get around the difficulties of low light circumstances during the early stages of growth when competition for sunlight is fierce (Mellerowicz and Gorshkova, 2012). The other ficus species, which make up the other half of the global ficus species, are gynodioecious (Rasplus et al., 2021). Gynodioecious ficus species are all native to the old world and thrives in harsh climatic circumstances like high temperatures and little water availability and grows abundantly without any agronomic treatment (Harrison et al., 2003; Swagel et al., 1997). Due to their strong therapeutic efficacy, higher plants have been widely employed as a source of many active ingredients for treating human ailments (Saeed et al., 2017; Weli et al., 2015).

The Assam rubber tree is indigenous to southern Indonesia and northeast India. India rubber tree is the name given to the plant locally (El-Baz and Hassan, 2017). *F. elastica* belongs to the family Moraceae commonly known as the rubber, rubber bush, rubber tree, rubber plant or Indian rubber bush, native to northeast India and southern Indonesia. The plant was first made available as an ornamental 100 years ago (Saeed et al., 2017). The plant is defined by its alkaloids, coumarins, triterpenes, steroids, flavonoids and tannins contents (El-Din et al., 2014). The plant is used by herbalists in West Africa to relieve musculoskeletal and articular pain (Mbosso et al., 2012). Rats that had arthritis and oedema caused by carrageenan was exposed to an aqueous extract of *F. elastica*, according to Sackeyfio and Lugeleka, (1986). The traditional usage of *F. elastica* in treating illnesses such skin infections, allergies, anaemia, neurodegenerative disorders and hepatic issues, as well as its use as a diuretic agent, led us to its selection for this study.

Depression is a state of low mood and an unwillingness to engage in activity. It can influence a person's motivation, feelings, ideas, behaviour and sense of wellbeing (Cui, 2015). Sadness, trouble concentrating and thinking clearly, as well as a substantial change in food or sleep schedule, may be present. Dejection, despair and even suicidal thoughts can all be symptoms of depression. It may be long term or short term. Anhedonia, which describes a loss of interest or pleasure in those things that ordinarily bring individuals delight, is considered to be the primary sign of depression. Some mood disorders, such as major depressive disorder or dysthymia, can cause depressed mood as a symptom. This study is about the phytoconstituents of *F. elastica* leaves in different extracts. The extracts were evaluated against antidepressant and anxiolytic effects by using classical *in-vivo* animal model such as FST, TST and rotarod and elevated plus maze models (Figure 1).

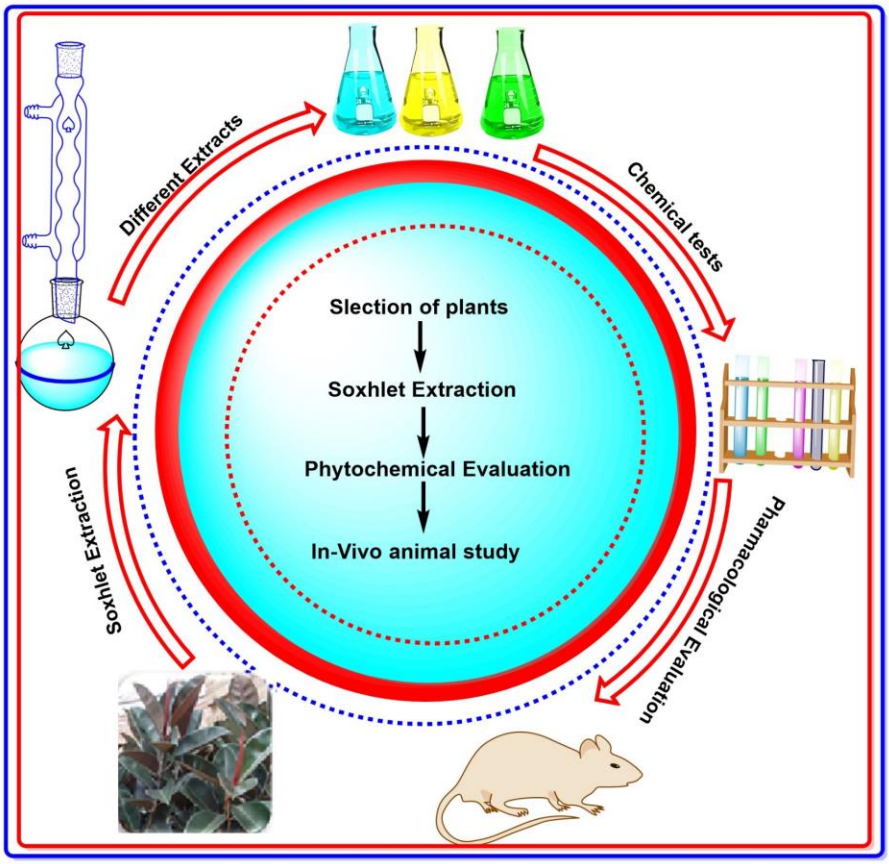

Figure 1 Workflow of the present study

2. METHODS

Plant Material

The fresh leaves of *Ficus elastica* were collected from the rural area of Howrah district in the month of May. The botanical identity of plant specimen authenticated by botanical survey of India, Botanical Garden, Howrah, India. The leaves were thoroughly washed, cleaned and shade dried properly for 10 days. The leaves were coarsely powdered and further utilised for preparation of chloroform, methanol and aqueous extracts.

Solvent extraction of plant material (leaves) and phytochemical screening

Extraction is the process of using a liquid solvent to remove active components from a solid or liquid, employing certain solvents to separate medicinally active parts of plant or animal tissues from inactive or inert parts (Ramalakshmi and Raghavan, 1999). In this process, the desired components are dissolved using menstruum, which are selective solvents and the remaining component is marc. Unwanted material is eliminated after the extraction.

Selection of solvent for soxhlet extraction

The selection of the solvent for soxhlet extraction is based on the phytoconstituents isolation process (Jones and Kinghorn, 2012). The solvent should be easy to remove and inert. Normally the solvent selection is based on the increasing polarity order like the order of petroleum ether, chloroform, ethyl acetate, ethanol, methanol, acetone and water (Chemat et al., 2019). Petroleum ether is frequently used for the extraction of steroids and fixed oils. It is also employed by certain researchers to defatten plant material. It is also used to remove chlorophyll from leaf powder. Defatting was followed by the use of a primary solvent, such as alcohol or water for extraction. Water is the polar solvent that is the least expensive and least poisonous, while methanol is the semi-polar solvent that can extract various phytoconstituents. Water isolates a number of polar compounds, making them useful for investigations on both humans and animals (Ali-Redha, 2021).

Soxhlet extraction

Dried leaves powder was weighed 15.25g and was extracted by Soxhlet apparatus at an elevated temperature of 65°C by using petroleum ether, chloroform, methanol and aqueous consecutively (De-Castro and Priego-Capote, 2010). The filtrates obtained were dried at a temperature of (40 ± 2)°C and gummy concentrated crude extracts was obtained. In the suitable container, the extracts were stored with proper labelling and maintaining storage conditions (Atiya et al., 2021).

Phytochemical Screening

Phytochemical screening of the crude extracts was carried out by employing standard procedures of different phytochemical constituents such as anthraquinones test, alkaloids (wagners's and dragendroff's test), carbohydrates, flavonoids, glycosides (liebermann's, keller-kiliani and salkowski's test), tannins (shinoda and alkaline reagent test), steroids and saponins (Adane et al., 2021).

Pharmacological Evaluation

All the pharmacological activities were conducted using swiss albino mice of either sex 25 -30g. They were acclimatized and housed in animal house with 12 hr: 12 hr light-dark cycle at 27 ± 2°C temperature and 45-55% relative humidity with sufficient food and water. The care and use of laboratory animals were strictly in accordance with the guidelines prescribed by the Institutional Animal Ethics Committee (IAEC). The work was approved by Calcutta Institute of Pharmaceutical Technology and Allied Health Sciences and CPCSEA with approval number 2075/PO/Re/S/19/CPCSEA.

Antidepressant activity

For the antidepressant activity, animals were divided into different five groups each contain 5 animals. Control group were treated with normal saline and test group were treated with standard drug fluoxetine (20 mg/kg) and test extracts of *Ficus elastica* (50 mg/kg) were dissolved in distilled water and administered orally once daily for seven days (Liu et al., 2019).

Forced Swimming Test (FST)

Antidepressant activity was screened by forced swim test (FST), according to the method described by Can et al., (2012), Petit-Demouliere et al., (2005) and Porsolt et al., (1977). Before starting the test, animals were trained 24h before. Trained animals were treated with the different extracts 50 mg/kg orally. The final four minutes of the six-minute testing session were used to record and immobility time was observed.

Tail Suspension Test (TST)

Tail suspension method was used for evaluation, according to Thierry et al., (1986) and Nomura et al., (1992). Adhesive tape was used to hold the mice 50 cm from the ground, around 1 cm from the tail tip. During a test period of 6 minutes, 4 minutes were measured for immobility. Only when mice hang passively and motionlessly were they deemed to be immobile.

Anxiolytic activity

For the anxiolytic activity, animals were divided into different five groups each contain 5 animals. Control group were treated with normal saline and test group were treated with standard drug diazepam (4 mg/kg) and test extracts of *Ficus elastica* (50 mg/kg) were dissolved in distilled water and administered orally once daily for seven days.

Elevated Plus Maze

Anxiety-like behaviour was measured using classical model named as elevated plus maze (40 cm length, 10 cm width and 50 cm height) (Kraeuter et al., 2019; Rodgers and Dalvi, 1997; Komada et al., 2008). The closed arms were enclosed by a black wall 20 cm in height. Each mouse was placed in the central area of the maze facing one of the open arms. Time spent in the open arms and closed arms were recorded for 300 seconds.

Rotarod activity

The neurotoxicity was measured, examined the mice by the rotarod screening test. The mice were previously trained to stay on an accelerating rotarod with specific dimensions (diameter 3.2 cm rotates at 10 rpm) (Deacon, 2013; Pal et al., 2022). Trained animals

were given *o.p.* test extracts at 50 mg/kg. Neurotoxicity was designated by the incapability of the animal to continue equilibrium on the rod for a minimum 1 min in each of the trials.

3. RESULTS

Phytochemical Tests

The yield of different extract of leaves of *Ficus elastica* was found in petroleum ether 0.78g, chloroform 0.44g, aqueous 0.87g and methanol 1.12g. The phytochemical tests were carried out by using chemical test (Table 1). Results described steroids and terpenoids were present in the extract of petroleum ether. In the extract of chloroform, alkaloids, flavonoids and terpenoids were parents. Methanol extract contains alkaloids, anthraquinones, tannins, flavonoids, glycosides and terpenoids whereas aqueous extract was able to elute out anthraquinones, tannins and terpenoids.

Table 1 Results of phytochemical tests of different extracts

Extract	Anthraquinones	Tannin	Alkaloid	Flavonoid	Glycoside	Terpenoid	Steroid
Petroleum ether	-	-	-	-	-	+	+
Chloroform	-	-	+	+	-	+	-
Methanol	+	+	+	+	+	+	-
Aqueous	+	+	-	-	-	+	-

Pharmacology

Antidepressant Activity

The antidepressant effects of aqueous, methanolic and chloroform extract of *Ficus elastica* (50 mg/kg) and Fluoxetine (20 mg/kg) were studied by observing the changes in the duration of immobility in the two models: Forced swim test (FST) and Tail suspension test (TST). In both TST and FST, methanolic extract of *Ficus elastica* 50 mg/kg produced significant reduction ($p<0.05$) in the immobility period when compared with that of standard group animals that received only the vehicle. The results are tabulated (Table 2, 3).

In FST, the mean duration of immobility was significantly reduced after 1 hr and 2 hr in standard as compared to the normal saline. The decrease in immobility with methanolic extract was statistically significant compared with normal saline ($P < 0.05$) and found to be significant when compared to fluoxetine ($P < 0.05$) (Figure 2). Also, mean duration of immobility was significantly decreases in mice with aqueous extract & chloroform extract when compared to control and standard groups. In TST, Mean duration of immobility was significantly reduced after 1 hr and 2 hr in standard as compared to the normal saline. The decrease in immobility with methanolic extract was statistically significant compared with normal saline ($P < 0.05$) and found to be significant when compared to fluoxetine ($P < 0.05$) (Figure 3). Also, mean duration of immobility was significantly decreased in mice with aqueous and chloroform extract when compared to standard and control groups. Immobility was significantly decreased in mice with aqueous and chloroform extract when compared to standard and control groups.

Table 2 Result of antidepressant activity in FST model of *Ficus elastica* leave extracts

Groups	Control		Fluoxetine (20mg/kg)		Aqueous Ext.(50mg/kg)		Methanolic Ext.(50mg/kg)		Chloroform Ext.(50mg/kg)	
Time interval	1 hr.	2 hr.	1 hr.	2 hr.	1 hr.	2 hr.	1 hr.	2 hr.	1 hr.	2 hr.
Mean ±SEM	221 ± 3.22	221.66 ± 4.33	137.33 ± 11.46*	122 ± 15.88*	103.66 ± 5.93*	78.33 ± 3.76*	75.66± 3.38**	52.66 ± 2.73**	102.66 ± 2.60*	84.66 ± 2.96*

*Significance < 0.05 as compared to control

Table 3 Result of antidepressant activity in TST model of *Ficus elastica* leave extracts

Groups	Control		Fluoxetine (20mg/kg)		Aqueous Ext.(50mg/kg)		Methanolic Ext.(50mg/kg)		Chloroform Ext.(50mg/kg)	
Time interval	1 hr.	2 hr.	1 hr.	2 hr.	1 hr.	2 hr.	1 hr.	2 hr.	1 hr.	2 hr.
Mean±SEM	251.66 ± 6.01	252.33 ± 5.36	196.33 ± 8.57*	173 ± 5.69*	121.33 ± 1.86**	109 ± 3.22**	91.0 ± 13.12**	71.66 ± 14.4**	143.33 ± 12.02*	124.3 ± 19.14*

*Significance < 0.05 as compared to control

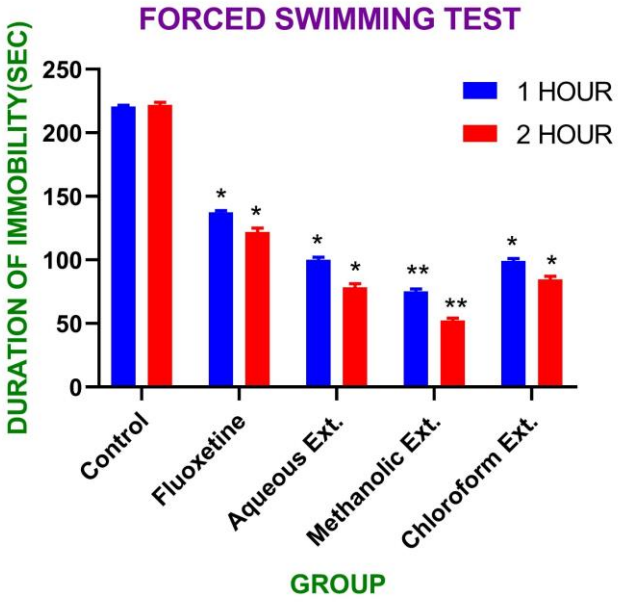

Figure 2 Effect of treatment of mice with controlled, fluoxetine (20mg/kg), aqueous, methanolic and chloroform extract (50mg/kg) of *Ficus elastica* leaves given orally in forced swim test (FST). Significant compared with normal saline ($P < 0.05$) and found to be significant when compared to fluoxetine ($P < 0.05$). Statistical values were obtained using one way ANOVA followed by Dunnet's test

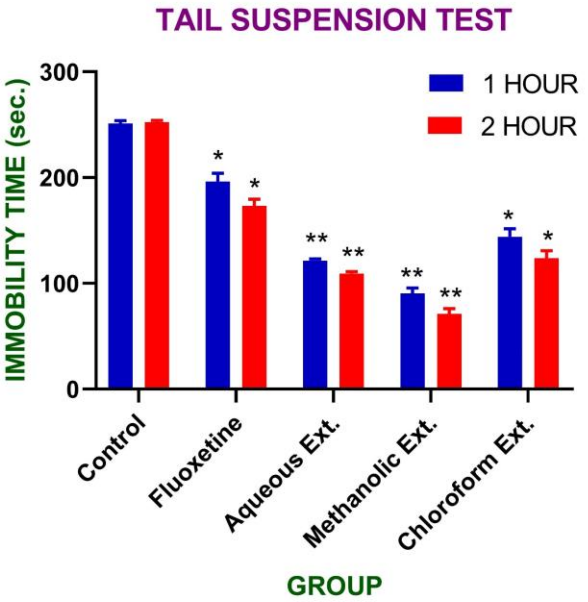

Figure 3 Effect of treatment of mice with controlled, fluoxetine (20mg/kg), aqueous, methanolic and chloroform extract (50mg/kg) of *Ficus elastica* leaves given orally in tail suspension test (TST). Significant compared with normal saline ($P < 0.05$) and found to be significant when compared to fluoxetine ($P < 0.05$). Statistical values were obtained using one way ANOVA followed by Dunnet's test

Anxiolytic activity

Elevated plus maze

Time sent by animal groups in open represents effectiveness of drugs whereas time spent by animal groups represents state of anxiety (stress, fear, panic and doom state) (Figure 4). The results demonstrated that chloroform extract was potent with

comparable time spent in open arm (97.28 sec) with standard (106.53 sec) (Table 4). The methanol extract was also showed noteworthy activity 72.71 sec of duration spent in open arm.

Table 4 Result of anxiolytic activity in elevated plus maze model of *Ficus elastica* leave extracts

S. No.	Animal Groups	Time spent in open arms (sec)	Time spent in closed arms (sec)
1	Control	16.61 ± 1.96	283.38 ± 1.96
2	Chloroform Ext	97.28 ± 3.70	202.62 ± 3.60
3	Methanol Ext	72.71 ± 4.62	227.29 ± 4.62
4	Aqueous Ext	42.45 ± 3.93	257.55 ± 3.94
5	Standard (Diazepam)	106.53 ± 4.35	193.47 ± 4.35

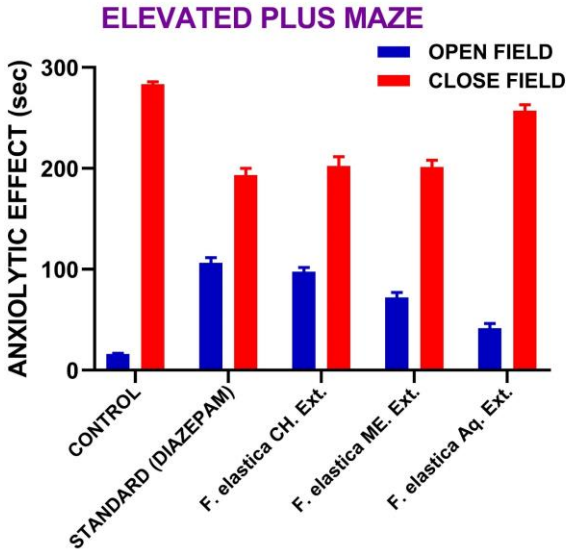

Figure 4 Effect of treatment of mice with controlled, diazepam (20mg/kg), aqueous, methanolic and chloroform extract (50mg/kg) of *Ficus elastica* leaves given orally in elevated plus maze model. Statistical values were obtained using one way ANOVA followed by Dunnet's test

Rotarod (Neurotoxicity)

Rotarod test is used to evaluate motor impairment. After 7th day animals were evaluated for rotarod test. The results revealed that none of the exact was responsible for neurotoxicity. All the animals were within the range of motor impairment when compared to the standard. The result for the neurological toxicity evaluation is given (Table 5).

Table 5 Result of rotarod test for neurotoxicity on 7th day

Group	0.9% NaCl	Standard (Diazepam)	Aqueous extract	Methanol extract	Chloroform extract
Mean ± SEM	165 ± 2.91	290 ± 2.79	270 ± 2.87	258 ± 3.06	254 ± 4.04

4. DISCUSSION

F. elastica is a member of Moraceae family which is commonly called as the rubber, rubber bush, rubber tree, rubber plant or Indian rubber bush. *F. elastica* leaves contains number of active biological constituents. The phytochemical screening described that *F. elastica* leaves are amalgamated with anthraquinones, tannins, alkaloids, flavonoids, glycosides, terpenoids and steroids. The different extractions of leaves were examined for neurological disorder models for depression and anxiety. After the extraction in petroleum ether, chloroform, aqueous and methanol, the amount of extract obtained was 0.78g, 0.44g, 0.87g and 1.12g respectively. The antidepressant activity of the extract was checked through two animal models of swiss albino mice i.e., FST and TST. In FST

and TST model for antidepressant like activity, methanolic extract was found to be most potent with significant decreased in immobility time.

In FST the methanolic extract (50 mg/kg) decreased the immobility time to 75.66 ± 3.38 second, whereas the standard fluoxetine was 137.33 ± 11 second after 1 hr. of action time. In TST the methanolic extract (50 mg/kg) decreased the immobility time to 91.0 ± 13.12 second, whereas the standard fluoxetine (20 mg/kg) was 196.33 ± 8.57 second after 1 hr. of action time. Elevated plus maze test was utilized for the evaluation of anxiolytic activity of the extracts and chloroform extract was found to be significant as compared to diazepam. In the chloroform extract the time spent on open arm was increased to 97.28 ± 3.70 second where as in diazepam it was found as 106.53 ± 4.35 second. Additionally, all extracts were also examined against rotarod test for the evaluation of locomotion activity. The results revealed that none of the extracts showed neurological toxicity and deflect of loco motor activity.

5. CONCLUSION

After all the above test and studies, it can be concluded that, *F. elastica* consist of active constituents which are responsible for the effectiveness against neurological disorders. The plant leaves contain anthraquinones and tannins in both methanol and aqueous extracts, alkaloids and flavonoids in both chloroform and methanol extracts, glycosides in methanol extract, terpenoids in all extracts, and steroids in petroleum ether extract. The different parts of *F. elastica* can also be evaluated for the assessment. The FST and TST study suggested that methanol extract of plant have potent activity of antidepressant activity and the elevated plus maze model revealed the anxiolytic activity of the same. The result also encourages for the isolation of phytoconstituents from different ficus species and evaluation for the neurodegenerative diseases.

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List of Abbreviations

CNS: Central Nervous System
WHO: World Health Organisation
FST: Forced Swim Test
TST: Tail Suspension Test
F. elastica: *Ficus elastica*
IAEC: Institutional Animal Ethics Committee

Informed consent

Not applicable.

Ethical approval

The work was approved by Calcutta Institute of Pharmaceutical Technology and Allied Health Sciences and CPCSEA with approval number 2075/PO/Re/S/19/CPCSEA.

Conflicts of interests

The authors declare that there are no conflicts of interests.

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Data and materials availability

All data associated with this study are present in the paper.

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